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Experimental paper

The neuroprotective effects of intraperitoneal injection of hydrogen in rabbits with cardiac arrest☆,☆☆

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ABSTRACT

Objective: The purpose of this study was to investigate the neuroprotective effects of intraperitoneal injection of hydrogen (H_2) in rabbits with cardiac arrest (CA).
Methods: A rabbit model of CA was established by the delivery of alternating current between the esophagus and chest wall to induce ventricular fibrillation. Before CA, the animals were randomly divided into four groups: a sham group (no CA), a CA group, a CA + low dose (10 ml/kg) H_2 group (CA + H_2 group 1), and a CA + high dose (20 ml/kg) H_2 group (CA + H_2 group 2). In the first experiment, animals were observed for 72 h after the restoration of spontaneous circulation (ROSC). The neurological scores were assessed at 24, 48 and 72 h after ROSC. The rabbits that survived until 72 h were sacrificed using an overdose of anesthetic, and the brain tissues were collected and Nissl-stained to observe nerve cell damage in the hippocampal CA1 area. In addition, TUNEL assay was performed to detect apoptosis. In the second experiment, animals were observed for 6 h after ROSC. Blood samples and brain hippocampal tissues were collected, and differences in oxidative stress indicators were compared among the four groups.
Results: Intraperitoneal injection of H_2 improved the 72-h survival rate and neurological scores, reduced neuronal injury and inhibited neuronal apoptosis. Intraperitoneal injection of H_2 reduced oxidative stress indicators in the plasma and hippocampal tissues and enhanced antioxidant enzyme activity. No significant difference was observed between the two CA groups treated with different doses of H_2.
Conclusions: Intraperitoneal injection of H_2 is a novel hydrogen administration method and can reduce cerebral ischemia-reperfusion injury and improve the prognosis of cardiopulmonary cerebral resuscitation in a rabbit model of CA.

1. Introduction

Cardiac arrest (CA) is a medical emergency that is a serious threat to human life. Recently, with the advances in cardiopulmonary resuscitation (CPR) techniques, a continuous rise in the rate of restoration of spontaneous circulation (ROSC) has been achieved in patients with CA.[1] However, the final survival rate remains very low, since only 7.9–8.5% of out-of-hospital patients with CA survive to hospital discharge.[2,3] Furthermore, about 50% of patients that survive to hospital discharge are complicated by moderate or severe neurological dysfunction. Therefore, enhancement of the success rate of CPR and reduction of impairment of cerebral function is still a hot topic for research in emergency medicine. Systemic ischemia-reperfusion injury is the most important pathophysiological process after CA, while oxidative stress and inflammation are the leading causes of systemic ischemia-reperfusion injury.[4] In 2007, inhalation of hydrogen (H_2) was shown to reduce cerebral infarct area and improve prognosis in a rat model of stroke,

Abbreviations: H_2, hydrogen; CA, cardiac arrest; ROSC, restoration of spontaneous circulation; CPR, cardiopulmonary resuscitation; HIS, immunohistochemical score; TUNEL, TdT-mediated dUTP nick-end labeling; 8-OHDG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; TBA, thiobarbituric acid; WST-1, water-soluble tetrazolium salt; CAT, catalase; SD, standard deviation; ANOVA, analysis of variance; LSD, least significant difference; •OH, hydroxyl radical; ROS, reactive oxygen species; ONOO-, peroxynitrite; O_2•, superoxide anion; Nrf2, Nuclear factor-E2 related factor 2; AMI, acute myocardial infarction.

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and H_2 was considered to act as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals.[5] Since then, H_2 has been demonstrated to protect tissues and organs from ischemia-reperfusion injury in several other animal models.[6–10] In addition to inhalation, some other administration techniques of H_2 have been developed, such as intravenous injection of H_2-rich saline,[10,11] intraperitoneal injection of H_2-rich saline[8,12] and drinking H_2-containing water.[7,13] In the present study, a rabbit model of CA was treated with intraperitoneal injection of H_2 to investigate the effect of H_2 on the prognosis of cardiopulmonary cerebral resuscitation.

2. Materials and methods

2.1. Animals

All animal experiments were performed in compliance with the *Guide for the Care and Use of Laboratory Animals* and approved by the Animal Ethics Committee of Sun Yat-Sen University (Institutional protocol number: [2012]146). New Zealand white rabbits were purchased from the Laboratory Animal Center of Sun Yat-Sen University and raised in individual cages.

2.2. Establishment of a CA animal model

A rabbit model of CA was established by the delivery of alternating current between the esophagus and chest wall to induce ventricular fibrillation. All animals were fasted but given free access to water on the night prior to the experiment. A 24-guage venous needle was used for right auricular vein puncture, and pentobarbital sodium (Sigma, USA) at a dose of 30 mg/kg was injected intravenously for anesthesia. The left ear artery was punctured using a 22-gauge venous needle to monitor arterial blood pressure. A BL-420s multichannel physiological signal recording system (Chengdu Taimeng Science and Technology Co., Ltd., Chengdu, China) was used to record the electrocardiogram and arterial blood pressure. A tracheal tube with internal diameter of 3.0 mm was inserted via the mouth using a blind endotracheal intubation method and fixed at a distance of 11 cm between the distal end of the tube and the incisor. A 30-guage acupuncture needle (0.30 mm × 2 mm) was inserted subcutaneously into the precordial region where the apical pulse was strongest, and esophageal pacing electrodes were implanted in the esophagus. The distance from the location of the metal ring of the electrodes to the incisor was 16 cm. We induced ventricular fibrillation with 35-mA constant current at a frequency of 50 Hz. CA was identified using the following criteria: (1) the systolic arterial pressure after electrical stimulation gradually fell to below 25 mmHg; and (2) pulsations in the arterial pressure waveform disappeared. After reaching the criteria for CA, electrical stimulation was performed for 1 min followed by 4 min of observation without treatment. After 5 min of CA, CPR was performed as previously described.[14] The indicators of ROSC included recovery of a supraventricular rhythm and a mean arterial pressure ≥60 mmHg that was sustained for >10 min. Animals without ROSC following standard cardiopulmonary resuscitation (CPR) for 15 min were defined as resuscitation failures.

2.3. Preparation of H_2

A M177021 hydrogen generator (Beijing Midwest Yuanda Technology Co., Ltd., Beijing, China) was used to produce pure H_2 (99.999% purity) by electrolysis of water. The H_2 produced was stored in aseptic soft plastic infusion bags and was used the same day when they were prepared.

2.4. Experimental design

Sixty-six New Zealand white rabbits were randomly assigned to one of four treatment groups: a sham group (no CA), a CA group, a CA + H_2 group 1, and a CA + H_2 group 2. The core temperature of the rabbits was continuously measured with a rectal temperature probe, which was maintained at 39 ± 0.5 °C using an infrared thermolamp until awake or 4 h after ROSC. Arterial and venous catheterization, anesthesia and endotracheal intubation were performed in the sham group. An esophageal electrode was implanted in the sham group with a length of 10 cm from the incisor, and then electrical stimulation using the same parameters was performed for 90 s to induce generalized twitching but not CA. In the three CA groups, ventricular fibrillation was induced for 5 min and then standard CPR was performed. Animals in the CA group were given an intraperitoneal injection of prewarmed physiological saline at a dose of 10 ml/kg 30 s prior to CPR; rabbits in CA + H_2 group 1 and CA + H_2 group 2 were given intraperitoneal injections of H_2 at doses of 10 and 20 ml/kg, respectively.

The first experiment was designed to investigate the effect of intraperitoneal injection of H_2 on survival rate and neurological function in rabbits with CA. Forty-two New Zealand white rabbits were randomly divided into the a sham group (6 rabbits), a CA group (12 rabbits), a CA + H_2 group 1 (12 rabbits) and a CA + H_2 group 2 (12 rabbits). The electrocardiogram and blood pressure was monitored for 4 h after ROSC in the CA group and the two CA + H_2 groups. During this period, those animals with weak spontaneous respiration underwent mechanical ventilation, but no other drugs were given. Respiration was assessed every 15 min to determine if further mechanical ventilation was necessary. Four hours later, mechanical ventilation was terminated. The tracheal tube was removed, and each rabbit was returned to its cage. The duration of mechanical ventilation after ROSC was defined as the period between the termination of CPR and the termination of mechanical ventilation. Rabbits that were still in a coma after 12 h of ROSC and could not drink water were given 5% glucose-saline at a dose of 1 ml/kg/h using a mini-pump. When the rabbits spontaneously drank water, the intravenous infusion was terminated. The survival duration after resuscitation was observed until 72 h. At 24, 48 and 72 h after ROSC, the neurological function of rabbits was scored using the 5-score evaluation method as previously described.[15] Scores 1 and 2 were considered to indicate a good prognosis of neurological function, whereas score 3 and greater were considered to indicate a poor prognosis. Those surviving to the termination of the observation period were sacrificed using an overdose of anesthetic. The brain was removed and fixed in 4% paraformaldehyde. Then, 3–5 mm thick brain tissues posterior to the optic chiasma in the coronal plane were cut and embedded in paraffin for pathological examination.

The second experiment was designed to assess the effect of intraperitoneal injection of H_2 on oxidative stress in rabbits with CA. Twenty-four rabbits were randomly assigned to one of the four treatment groups with 6 animals in each group. The observation period was terminated 6 h after CPR. Blood samples were collected before and 6 h after CPR, and centrifuged at 4000 rpm for 10 min. The plasma was collected and stored at −80 °C for subsequent analysis. After 6 h of observation, the rabbits were sacrificed using an overdose of anesthetic and perfused with 0.9% physiological saline at 4 °C via the heart. The brain was removed and the hippocampal tissues were isolated on ice. The hippocampus was mixed with physiological saline and ground at 4000 rpm for 10 min. The supernatant was 10% tissue homogenate, and this was stored at −80 °C for subsequent analysis.